

## DEDICATED DECONTAMINATION: A NECESSITY TO PREVENT CROSS CONTAMINATION IN HIGH THROUGHPUT MYCOBACTERIOLOGY LABORATORIES

\*C Rodrigues, D Almeida, S Shenai, N Goyal, A Mehta

### Abstract

Unrecognized cross-contamination has been known to occur in laboratories frequently, especially with sensitive recovery system like BACTEC 460 TB. In March 2001, we investigated a pseudo-outbreak of *Mycobacterium tuberculosis* isolates in three smear negative clinical specimens and would like to present our experience in this communication. **Methods:** All suspected cases were confirmed by checking the drug susceptibility and DNA fingerprints using spoligotyping as well as restriction fragment length polymorphism. **Results:** On investigation, the most likely cause was found to be the use of common decontamination reagents and phosphate buffer. **Conclusions:** To avoid erroneous diagnosis, we have devised a dedicated decontamination procedure, which includes separate aliquoting of phosphate buffer and decontamination reagents per patient. Timely molecular analysis and appropriate changes to specimen processing have been identified as useful measures for limiting laboratory cross contamination.

**Key words:** Cross contamination, dedicated decontamination, pseudo-outbreak

Cross-contamination is an inherent problem in culturing mycobacteria.<sup>1,2</sup> Unrecognized cross-contamination has been known to occur in laboratories frequently, especially with sensitive recovery system like BACTEC 460 TB system.<sup>3</sup> The potential impact of cross contamination results in false positive cultures which may lead to needless therapy with all its problems.

We report use of spoligotyping to confirm our suspicion that cross-contamination had occurred in our mycobacteriology laboratory, in the year 2001 and use of procedures such as dedicated decontamination for limiting such future instances.

### Materials and Methods

The mycobacteriology laboratory at PD Hinduja National Hospital and Medical Research Center, Mumbai, India, receives about 3500 requests for acid-fast bacteria cultures annually, 45% of which are positive for *M. tuberculosis* complex. All the specimens we receive are decontaminated by standard NALC-NaOH method<sup>4</sup> and then inoculated into BACTEC 12 B vial along with one Lowenstein & Jensen (L.J) slant.

In this episode, suspected cross contamination cases were confirmed by antimycobacterial drug susceptibility patterns, spoligotyping and restriction fragment length polymorphism (RFLP).

### Drug susceptibility testing

Antimycobacterial drug susceptibility of all the specimens was determined by BACTEC 460 TB system.

### Spoligotyping

Spoligotyping was performed with a commercially available kit (Isogen Bioscience). Briefly, the procedure involved amplification of the direct repeat (DR) region of *M. tuberculosis* genome by polymerase chain reaction using specific biotinylated primers.<sup>5</sup> The amplified products were then hybridized onto a nylon membrane blotted with 43 spacer sequences. The spacer sequences were specific to the DR region and the strains differed in presence or absence of these spacer sequences. The results of hybridization were detected by ECL detection system.<sup>6,7</sup>

### RFLP analysis

RFLP analysis was performed by the internationally standardized procedure.<sup>7</sup> In brief, bacterial cell walls were lysed and whole genomic DNA was extracted and digested with PvuII. The resulting DNA fingerprints were separated by gel electrophoresis, transferred to nylon membrane and probed with a horseradish peroxidase-labeled 245-bp sequence of IS6110 DNA.

### Clinical correlation

The medical records of all six patients were reviewed and the treating physicians were contacted for clinical details. The laboratory technician was interviewed to obtain information about specimen handling and processing. Confidentiality of all reports was maintained.

\*Corresponding author (email: <dr\_crodrigues@hindujahospital.com>)  
P. D. Hinduja Hospital and MRC, Veer Savarkar Marg, Mahim (West), Mumbai - 400 016, Maharashtra, India.

Received : 07-11-05

Accepted : 17-10-06

## Results

A retrospective investigation was carried out after receiving the feedback of a report of *M. tuberculosis* complex in a patient not clinically suspected to have tuberculosis (TB). Review of laboratory records revealed that of the nine consecutive specimens processed in the same batch, six were positive by culture (Table 1). Of these six specimens, three were acid-fast bacilli (AFB) negative in smears, which included a urine sample, a bronchoalveolar lavage and a pus sample. All these three cultures were positive only in the BACTEC bottles almost after three weeks and yielded no growth on the accompanying LJ slants. Clinical findings for these three patients were not consistent with active TB. The drug susceptibility testing indicated multidrug resistance i.e., resistance to isoniazid and rifampicin.

All the six *M. tuberculosis* isolates were subjected to spoligotyping. Result analysis showed the identical spoligopatterns in four out of six *M. tuberculosis* isolates. Review of laboratory records revealed that, of the four clinical specimens showing identical spoligotypes, one was smear positive and considered to be the index specimen. Remaining two isolates grown from smear positive specimens had different spoligotypes hence were considered true positives.

Since spoligotyping is known to be less refractory for differentiation of *M. tuberculosis*, we also analyzed the strains using IS6110 RFLP analysis. However, only three strains (index specimen and two contaminated specimens) could be typed using RFLP due to lack of availability of adequate or good quality DNA. All these three specimens typed showed same RFLP pattern indicated by a single band.

## Discussion

Review of literature reveals a large variation in the reported rates of cross-contamination, ranging from 0.1 to 65%.<sup>8</sup> Multiple factors can contribute to cross contamination, including contaminated clinical devices, clerical errors and

human or technical errors that occur in the laboratory while processing clinical specimens. The last category can be caused by batch processing, laboratory protocols not adapted for left-handedness,<sup>8,9</sup> sleeve of the laboratory coats of technicians touching the tubes, use of common decontamination reagents and phosphate buffer, use of a faulty exhaust hood, soiled needles or faulty needle heater circuit in the BACTEC 460 TB system.<sup>1,3,8,10</sup>

In this series, all the specimens were processed in the biological safety cabinet class II by general laboratory technician. However, the order of processing was neither according to the accession number nor in the ascending order of bacterial load. The AFB smear positive sputum samples were processed before the smear negative samples. Further investigation suggested that the cross-contamination probably resulted while dispensing decontamination reagents and neutralizing agent [phosphate buffered saline (PBS)] with a common flask. PBS was prepared in a liter quantity and the contamination might have occurred due to unintentional touching the edges of the open specimen containing tubes with the PBS flask or droplets splashed from a sample in a specimen containing tube while pouring PBS directly through the heavy flask. Since this pseudo-outbreak was recorded almost three weeks after the inoculation, the original decontamination reagents and phosphate buffer were not available for culture.

Recognition of laboratory cross-contamination and elucidation of its probable cause has resulted in the revision of laboratory procedures. Instead of using general laboratory technicians, we have two full-time, thoroughly trained and experienced TB-technician dedicated to mycobacteriology. In addition two more technicians are trained for emergency back-up. To avoid cross-contamination we have devised a dedicated decontamination procedure for decontamination as given below:

1. Separate aliquoting of PBS and decontamination reagents

**Table 1: Details of nine consecutive specimens processed in the same batch**

Specimen	Acc. no.	Acid-fast bacilli smear	Culture		Drug resistance
			TB- BACTEC	LJ medium	
Urine	530	Negative	MTB (3 wk)	NG	R - IR
BAL	531	+++	MTB (19 days)	MTB	R - SIREEtP
Sputum	533	++	MTB (15 days)	MTB	R - IR
Sputum	535	Occasional	Nocardia	NG	
BAL	536	Negative	MTB (3 wk )	NG	R -IR
Pus	537	Negative	MTB (3 wk)	NG	R - IR
Pleural fld	539	Negative	NG	NG	
Abscess	540	Negative	MTB (3 wk)	MTB	S-SIREKEtPOZ
Sputum	541	Negative	NG	NG	

MTB- *M. tuberculosis* complex, NG- No growth, R- Resistant, S- Sensitive, BAL-Bronchoalveolar lavage, Fld- fluid, Drugs : S- Streptomycin, I – Isoniazid, R- Rifampicin, E- Ethambutol, K- Kanamycin, Et- Ethionamide, P- Para amino salicylic acid, O- Ofloxacin, Z- Pyrazinamidase  
Note: All specimens are listed as per the accession number; however, the exact order of processing was not known.

per patient. All the decontamination reagents i.e., N - acetyl - L - cysteine solution, 4% NaOH, 2.9% Na - citrate, 0.067 M Phosphate buffer ph 6.8 are freshly prepared daily and aliquoted in a smaller glass containers, autoclaved and then bar-coded with patient's identification. As all these containers can be washed, autoclaved and reused, the method is cost-effective.

2. All specimens obtained from sterile body sites are centrifuged and cultured without decontamination.
3. We ensure that the order of specimens processing is from low bacterial load to high bacterial load. Extrapulmonary specimens especially cerebrospinal fluid (CSF) are always processed first. Acid-fast positivity (smear result) is provided within two hours to the healthcare provider.
4. As a general rule only one tube is opened at a time and remaining tubes are kept at least 12 cm away from aerosol-generating activities to minimize the potential for cross contamination.

In addition, we have started with a regular follow-up of all AFB culture positive patients and a timely inspection of all *M. tuberculosis* isolates grown in BACTEC 460 TB system by spoligotyping to validate our results. We are following these revised laboratory procedures for the past four years and the incidence of false positive cultures was not repeated again suggesting that the dedicated decontamination did play a role in eliminating the problem.

Identification of cross-contaminated cultures has important consequences such as erroneous diagnosis and needless therapy. The laboratories may also be able to identify certain equipment or procedural errors leading to false positive cultures and if corrected promptly may minimize erroneous reporting. We believe that the use of dedicated decontamination is useful in minimizing cross contamination. Regular clinical feedback is vital in identifying potential cases of cross-contamination. Use of simple and rapid molecular methods aid in diagnosis of laboratory cross-contamination.

## References

1. Burman WJ, Stone BL, Reves RR, Wilson ML, Yang Z, El-Hajj H, *et al.* The incidence of false-positive cultures for *Mycobacterium tuberculosis*. *Am J Respir Crit Care Med* 1997;**155**:321-6.
2. de C Ramos M, Soini H, Roscanni GC, Jaques M, Villares MC, Musser JM. Extensive cross-contamination of specimens with *Mycobacterium tuberculosis* in a reference laboratory. *J Clin Microbiol* 1999;**37**:916-9.
3. Burman WJ, Reves RR. Review of false-positive cultures for *Mycobacterium tuberculosis* and recommendations for avoiding unnecessary treatment. *Clin Infect Dis* 2000;**31**:1390-5.
4. Kent T, Kubica GP. Public health mycobacteriology a guide for level III lab. U.S. Department of health and human services, Public health services. Center for Disease Control: Atlanta; 1985.
5. Kamerbeek J, Schouls L, Kolk A, van Agterveld M, van Soolingen D, Kuijper S, *et al.* Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. *J Clin Microbiol* 1997;**35**:907-14.
6. Groenen PM, Bunschoten AE, van Soolingen D, van Hafner D. Nature of DNA polymorphism in the direct repeat cluster of *Mycobacterium tuberculosis*: Application for strain differentiation by a novel typing method. *Mol Microbiol* 1993;**10**:1057-65.
7. van Embden JD, Cave MD, Crawford JT, Dale JW, Eisenach KD, Gicquel B, *et al.* Strain identification of *Mycobacterium tuberculosis* by DNA fingerprinting: Recommendations for a standardized methodology. *J Clin Microbiol* 1993;**31**:406-9.
8. Ruddy M, McHugh TD, Dale JW, Banerjee D, Maguire H, Wilson P, *et al.* Estimation of the rate of unrecognized cross-contamination with *Mycobacterium tuberculosis* in London Microbiology Laboratories. *J Clin Microbiol* 2002;**40**:4100-4.
9. Bauer J, Thomsen VO, Poulsen S, Andersen AB. False-positive results from cultures of *Mycobacterium tuberculosis* due to laboratory cross-contamination confirmed by restriction fragment length polymorphism. *J Clin Microbiol* 1997;**35**:988-91.
10. Small PM, McClenny NB, Singh SP, Schoolnik GK, Tompkins LS, Mickelsen PA. Molecular strain typing of *Mycobacterium tuberculosis* to confirm cross-contamination in the mycobacteriology laboratory and modification of procedures to minimize occurrence of false-positive cultures. *J Clin Microbiol* 1993;**31**:1677-82.

**Source of Support:** Nil, **Conflict of Interest:** None declared.